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Evaluation of Rhizobial Strain of Faba Bean for Symbiotic Performance Grown in Acid-Prone Areas, Guagusa Shekudad District

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Abstract: Faba bean (Vicia faba L.) is a legume crop grown primarily for its edible seeds (beans) and a major legume seed consumed by humans worldwide. So as boost its productivity, application of naturally symbiotic fertilizer is essential. Hence, the objective of this trial was to evaluate rhizobia naturally associated with faba bean for acidic soil. In order to deadline the mentioned goals, different nodule data and yield attributing traits were taken and evaluated based on standard procedure. Accordingly, the highest nodule number (151 per plant) were obtained when the faba bean were inoculated with rhizobia isolate FB-EM-05 in year 1 and 40.7 per plant were obtained when there were no any inoculations of Faba bean seed in year 2. Additionally the highest nodule dry weight 1.1 and 0.68 gram per plant were obtained when the faba bean isolate were inoculated with FB-EM-05 and FB-NS-03 respectively in 2019. The highest biomass per hectare 11111.11kg were obtained when the seeds treated with rhizobia isolates FB-NS-03. The highest grain yield 2883.69 kg per hectare were obtained from faba bean inoculated with isolate FB-NS-03 which increase the grain yield about 24.14 percent over the negative control in year 2019. However, the highest above ground biomass (4500 kg per hectare) among the rhizobia isolate was recorded from isolate FB-EM-02 and the highest grain yield was obtained from faba bean treated with isolate FB-EM-05 and yielded about 1532.3kg per hectare. Overall, the highest Faba bean grain yield was obtained when the seeds were treated with isolate FB-NS-03 account for the result of 2297 kilogram per hectare and will be used for biofertilizer production.

Keywords: Biofertilizer, Fababean, Isolate, Nodule

1. Introduction

Leguminous crops are crop type which has high advantages over other cereal crops. They cover most the agricultural land next to cereal crops. Fababean is the major beneficial crops for worldwide used as feed and foods, extensively ability to fix symbiotically fix and add nitrogen to the soil and increase soil fertility and thereby enhancing and sustaining soil productivity and minimize the farmers burden to use the chemical fertilizer [12] Among the legumes crops, soybean, fababean, cowpea, lupin and alphalpha are the known legume crops used for the maintenance of the replenished soil [5]. Due to its agro ecological properties, fababean are the highland legume crops which is mostly affected by soil acidity due to its high rainfall and accumulates huge amount of Al-toxicity and and phosphorous fixation [15] (Tadele, M. (2020). Those are where there is high accumulation of soil acidity; nitrogen fixation is almost none and brings the loss of high fababean yield production [4] Bordeleau, L. M., & Prévost, D. (1994).

Different practices experienced that, application of lime ameliorate the soil acidity [7] Fageria, N. K., & Baligar, V. C. (2008). However, this is not permanent and sustainable solution due to its large rate of application at single site. However, different scientists stated that, isolation of rhizobia which is host specific from acidic soil. According to the findings of [19] 23 kg of yield is increased over the control using acidic tolerant rhizobia isolates.

Although different research findings were explained the acceptable results, there was research gap around the study area. Hence, this proposal were planned to solve such challenges and evaluate rhizobia naturally associated with fababean in acid prone area.

2. Material and Methods

2.1. Study Area Description and Treatment Arrangement

The trials were conducted in in Guagusa-Shikudad district at rain fed condition in two consecutive years of 2019 and 2020 northern parts of Ethiopia. Guagusa shikudad is one of the districts in Amhara regional state of Ethiopia and is surrounded on the west by Ankasha Guagusa, the east and south by the Mirab Gojjam Zone, and the north by Banja Shekudad. It is a part of the Agew Awi Zone. The district is situated at altitude of the coordinates of 10°48′32″ north and 37°04′12″ east and 428 km away from Addis Ababa. The area has a uni-modal rain-fall pattern. The rainy months extend from March to the end of November. Rainfall during these months is erratic and has distribution challenges. However, peak rainfall occurs during the months of July and August. The soils in the area are predominantly Nitosol and some are of vertic properties [3] (ANRS-BoFED, 2006). The area is characterized by mixed farming system. Potato, wheat, barley, maize, field pea and faba bean are the dominant crops grown in the area. These crops cover around 90% of the cultivated area.

The activity was organized in randomized complete block design with three replication in each year and treatment arrangement was T1= Negative control (no input), T2= control (receiving only P), T3= Positive control (receiving N source T4= FB-NS-02, T5= FB-NS-03, T6=FB-EM-02, T7= FB-EM-05, T8=FB-EM-07, T9= FB-EM-11). The experimental inoculum was taken from Holeta Agricultural Research Center, soil Microbiology Laboratory.

2.2. Land Preparation, Inoculation and Other Agronomic Procedures

A test crop of which was high-yielding Gebelcho variety of Faba bean, which was frequently grown in Guagusashukidad district, was used. The trial site, which was free from inoculation history were selected. The experimental fields were prepared by oxen to make it ready for planting in end of May to early June. The working sites were then leveled and divided into blocks and each plots. Blocks were spaced out 1.5 meters apart, with the plots preserved at 1 meter apart. Each plot's beds were created by preparing canals all around it. Each strain's carrier-based inoculants were applied at a rate of 10 g inoculant/kg seed, procured from Holeta Agricultural Research Center. The needed amount of inoculant was suspended in a 1:1 mixture with 10% sugar solution to guarantee that all of the applied inoculum adhered to the seed. Dry seeds were carefully combined with the thick slurry of the inoculants so that each seed received a thin coating of the inoculants. To maintain the viability of bacterial cells, all inoculations were completed right before planting in the shade. After a brief period of air drying, seeds were sowed with the necessary rate and spacing. To prevent contamination, not inoculated seeds were sown first. After being drilled, the infected seeds were planted at a distance of 10 cm between plants and 40 cm between rows. To prevent cell death from sunlight heat, the soil was immediately covered after sowing. After two weeks, the plants were thinned. All the agronomic management practices were done uniformly for all experimental units.

2.3. Data Collection Methods

Five randomly chosen second border rows of each plot's flowering plants were used to collect data on nodulation (nodule number and nodule dry weight). To determine the precise nodule numbers and nodule dry weight, five plants were carefully removed at random from each plot using a spade. The soil that stuck was gotten rid of by gently running water over a metal sieve over the roots of any intact nodules. The number of nodules per plant was calculated by adding the average number of nodules from each of the five uprooted plants in the plot. When all five plant samples from each plot had been gathered, the nodules were combined, and their dry weight was calculated by drying them at 70°C for 72 hours to a consistent weight. The dry weight was then reported as g/plant.

At maturity, each plot's fababean plants were removed after they reached physiological maturity, leaving only the two boundary rows on each plot. Using the formula provided by Abebe [1] (1979), the moisture content was adjusted to 12.5% to get the desired seed yield and converted to kilogram per hectare.

Adjusted grain yield $= \frac{(100-\mathrm{MC})}{100-12.5} *$ grain yield; Where MC is moisture content of fababean seeds at the time of measurement, and 12.5 is the standard moisture content of faba bean seeds at harvest in percent.

2.4. Data Analysis Methods

Using statistical analysis systems (SAS) software, the obtained data were subjected to analysis of variance (ANOVA) in a Randomized Complete Block Design (RCBD) that was duplicated three times [18]. Fisher's test with a 0.05 level of significance was used to compare treatment means using the general linear model (GLM).

3. Result and Discussion

3.1. The Effects of Different Faba Bean Rhizobia Isolates on Nodule Parameters on (2019)

The faba bean nodule number and dry weight were affected by different rhizobia isolates (Table 1). According to the result obtained, the highest nodule number (151 per plant) were obtained when the faba bean were inoculated with rhizobia isolate FB-EM-05. But, there were statistically no significance difference with most rhizobial isolates. This results indicated that inoculation of those isolates with faba

bean seeds play crucial role in increasing faba bean nodule number than the naturally existing rhizobia in the soil. According to the report of [17] Samudin, S., & Kuswantoro, H. (2018) the significant effect of inoculation was shown by the number of nodules, nodule dry weight, root length, and root dry weight. This result in in line with the finding of [9] who reported that rhizobia isolate brought significance difference on nodulation number of faba bean when inoculated with faba bean seeds than the naturally existing rhizobacteriam in the soil. In the same manner, [6] (Chimdi A., et al., 2022) also reported that the maximum number of nodules per plant for 73.9 was recorded from the plots treated by strain Fb_{18}. The lowest nodule number per plant 97.73 was obtained when the seeds were inoculated with FB-EM-07. The similarity results obtained from all treatments might be the presence of potential native rhizobia in the soil at Farmer Abere farm land in 2019.

The nodule dry weights per plant were also influenced by different rhizobia isolate. The highest nodule dry weight 1.1 gram per plant were obtained when the faba bean isolate were inoculated with FB-EM-05. Even though numerically different results were obtained in the trial, statistically no significance difference results were obtained from almost treatments. However, the lowest nodule dry weight was obtained from seed inoculated with isolate FB-EM-07 account for 0.43 gram per plant. The result is in line with the findings of Woldekiros, B., et al., [16], stated that inoculation of rhizobium strain significantly affected all parameters studied in this experiment except number of seeds pod-1.

3.2. The Effects of Different Rhizobia Isolates on Above Ground Biomass and Grain Yield on (2019)

In this study, the highest biomass per hectare 11111.11kg were obtained when the seeds treated with rhizobia isolates FB-NS-03. Similarly, almost same biomasses were registered from all isolates. However, the lowest biomass yield (7430.56 kg) was obtained from negative control which has no any input in the treatment (Table 1). In spite of the fact that no significance difference were exist among all the treatments, the highest grain yield 2883.69 kg per hectare were obtained from faba bean inoculated with isolate FB-NS-03 which increase the grain yield about 24.14 percent over the negative control and have no significance difference with isolate coded by FB-EM-02. contrary to this result, the lowest grain yield was obtained from seeds inoculated with isolate FB-EM-05 which account for 2126.65 kg per hectare and brought about 2.86 percentage below the negative control. According to the findings of [10], inoculation of faba bean with rhizobia isolates could significantly affect the all parameters and hence boost the grain yield and biomass of the crop. According to his conclusion, inoculated plants gave the highest yield advantage compared to un-inoculated. In the same way [2], reported that, the main effects of inoculation and phosphorus application significantly ($P \leq 0.01$) influenced the yield. Inoculating faba bean seed with FB-1035 rhizobium isolate resulted in the highest grain yield of 3137 kg ha^{-1}.

Table 1. *Nodule parameters and grain yield of faba bean as influenced by Rhizobium inoculation in Guagusa shukidad (2019).*

Treatments	Nodule number/plant	Nodule Dry weight (g/plant)	Biomass weight (kg/ha)	Grain Yield (kg/ha)	GY increment Over control in %
1. –Ve-control	113.07	1.03a	7430.56b	2187.46	0.00
2. TSP	150.8	1.10a	10486.11ab	2550.94	14.25
3. TSP+Urea	142.4	0.93ab	10000.00ab	2654.35	17.59
4. FB-NS-02	131.47	0.97a	9305.56ab	2699.31	18.96
5. FB-NS-03	112.47	0.97a	11111.11a	2883.69	24.14
6. FB-EM-02	144.67	0.93ab	10347.22ab	2881.59	24.09
7. FB-EM-05	151	1.10a	8055.56ab	2126.65	-2.86
8. FB-EM-07	97.73	0.43b	10277.78ab	2636.32	17.03
9. FB-EM-11	141.73	0.80ab	8958.33ab	2612.83	16.28
lsd (p=0.05)	ns	0.51	3353.2	Ns	-
cv (%)	30.21	20.46	20.46	20.79	-

LSD= least significance difference at probability of 0.05, CV =coefficient of variation in percentage

3.3. Effects of Faba Bean Nodule Parameters as Affected by Different Rhizobia Isolates (2020)

In the year 2020, the activity was laid out on another farm and the different parameters were slightly affected by rhizobia isolates (Table 2). Even though there were no significance differences among all treatments, the numbers of nodules per plant were different from isolate to isolate. Accordingly, the highest nodule number per plant (38.7) was obtained when the seeds were inoculated with FB-NS-03 and the lowest nodule number was obtained when the faba bean seed inoculated with isolate FB-EM-05 which was account for 17.8. Among the all treatment, the highest nodule numbers 40.7 per plant were obtained when there were no any inoculations of faba bean seed. This might be there was potential native rhizobia isolate which could tolerate high acidic soil. In the same manner, the highest (0.68 per plant) and the lowest nodule dry weight (0.27) were obtained when the seeds were inoculated with isolate FB-NS-03 and FB-EM-07 respectively. According to the experimental findings, four isolates (FB-NS-02, FB-NS-03, FB-EM-02 and FB-EM-11) were almost equally affect the nodule dry weight numerically and statistically have no significant different among them. Overall, the highest nodule dry weight was obtained when the experimental unit was treated with

chemical fertilizer TSP+Urea account for 0.7 gram per plant whereas, the lowest nodule dry weight (0.25 gram) was from untreated treatment or negative control (Table below). In the same manner, Similar results was reported by [11], who stated that the total number of nodules per plant of faba bean was non-significantly ($p< 0.05$) affected by the Rhizobium strains alone and with TSP fertilizer. The maximum number of nodules per plant (29) was recorded from the treatment FB 1018 while the minimum number (21) was recorded from control treatment.

Table 2. *Nodulation and yield traits of faba bean as affected by rhizobium inoculation (Abebayehu 2020).*

Treatments	Nodule number/plant	Nodule Dry weight (g/plant)	Biomass weight (kg/ha)	Grain Yield (kg/ha)	GY increment Over control in %
1. –Ve control	40.7	0.25c	4333.3	913.9	0.00
2. TSP	37.4	0.55abc	5500.0	1255.8	27.23
3. TSP+Urea	8.8	0.7a	4583.3	1494.9	38.87
4. FB-NS-02	28	0.36abc	4333.3	1509.3	39.45
5. FB-NS-03	38.7	0.68ab	3750.0	1417.6	35.53
6. FB-EM-02	19.2	0.34abc	4500.0	1355.3	32.57
7. FB-EM-05	17.8	0.31bc	4166.7	1532.3	40.36
8. FB-EM-07	19.5	0.27c	4500.0	1251.8	26.99
9. FB-EM-11	28.5	0.58abc	4166.7	1058.0	13.62
lsd (p=0.05)	ns	0.36	ns	Ns	-
CV (%)	24.11	34.7	26.2	34.26	-

LSD= least significance difference at probability of 0.05, CV =coefficient of variation in percentage

3.4. Effects of Faba Bean Above Ground Biomass and Grain Yield as Affected by Different Rhizobia Isolates

Although there are no significance difference among all treatments based on above ground biomass, the highest above ground biomass (4500 kg per hectare) among the rhizobia isolate was recorded FB-EM-02 and FB-EM-07 followed by isolate FB-NS-02 which was 4333.3 kilogram per hectare (Table 2). But, the lowest above ground biomass was obtained from faba bean inoculated with isolate FB-NS-03 among all isolates and statistically no significance difference from the other at P=0.05. The yield of faba bean above ground biomass difference among the isolates might be the potential of each isolate varies among them in fixing atmospheric nitrogen biologically for addition of nitrogen to the plant. In the same manner, [11], observed that the different types of Rhizobium strains alone and with TSP fertilizer had a significant (It was observed that the different types of Rhizobium strains alone and with TSP fertilizer had a significant (($p<0.05$) influence on the aboveground biomass production. Overall comparison, the highest biomass (5500.0kg/ha) was gotten when the Triple supper phosphate added to the plot followed by the one treated with Triple super phosphate and Urea account for 4583.3 kilogram per hectare.

The faba bean grain yield was also influenced by rhizobia isolates as indicated in Table 2. Accordingly, the highest grain yield was obtained from faba bean treated with isolate FB-EM-05 and yielded about 1532.3kg per hectare. Although this isolate have no significance difference among most isolates, it had about 40.36 percentages of yield increment over the negative control. According to the finding [8] analysis of variance indicated that, faba bean grain yield was highly affected by the treatment of 50Kg/ha NPS Kg/ha FB EAL-1017 rhizobial strains and significantly ($P \leq 0.05$) influenced faba bean grain yield.

In contrary to this results, the lowest grain yield per hectare (1058.0) were recorded when the faba bean seeds were inoculated with isolate FB-EM-11. However, this result was superior on yield advantages over the negative control nearly by (13.62) percentage (Table 2). According to the report Lishan, T., et al., [13] the highest grain yield (2540 kg/ha) was obtained from the application of 0.75 kg/ha bio-fertiliser and 50 cm inter-row spacing while the lowest (1083 kg ha) was obtained from the control of no bio-fertiliser. Similarly, [14] reported that the highest grain yields (3101.4 kg ha-1 and 2182.5 kg ha-1) were obtained from inoculation with rhizobial isolate FB-17 in Welmera and Ejere districts and Seed inoculation of faba bean with rhizobial isolates showed significant ($p\leq 0.05$) difference on grain yield of faba bean.

Table 3. *Over year over location combined analysis result of fababean rhizobia strain.*

Treatments arrangement	Nodule number/plant	Nodule Dry weight (g/plant)	Biomass weight (kg/ha)	Grain Yield (kg/ha)
1. –Ve control	84	0.72	6191	1678
2. TSP	105	0.88	8492	2033
3. TSP+Urea	89	0.84	7833	2191
4. FB-NS-02	90	0.72	7317	2223
5. FB-NS-03	83	0.86	8167	2297
6. FB-EM-02	94	0.70	8008	2271
7. FB-EM-05	98	0.78	6500	1889
8. FB-EM-07	66	0.36	7967	2082

Treatments arrangement	Nodule number/plant	Nodule Dry weight (g/plant)	Biomass weight (kg/ha)	Grain Yield (kg/ha)
9. FB-EM-11	96	0.70	7042	1991
LSD (p=0.05)	ns	ns	ns	ns
CV (%)	37	36.70	22.3	22.3

Key: CV coefficient of variation, LSD= least significance difference, ns= non-significant

On both years even though there was not any significant difference of grain yield and biomass yield among the treatments, the highest Faba bean grain yield was obtained when the seeds were treated with isolate FB-NS-03 account for the result of 2297 kilogram per hectare. In terms of above ground biomass, faba bean seed inoculated with isolate FB-EM-02 yielded high biomass per hectare and there were no significance difference with isolate FB-NS-03. One reason may be there will be effective native rhizobial strains in the soil. In 2020 the overall grain yields also lower than expected yield. This may be due to high acidity of the working site.

4. Conclusion

Application of bio-inoculunt play a crucial role for sustainable agriculture and this technology improve soil fertility and minimize the usage of synthetic fertilizer. Even though numerous researches were performed at different soil types, the problem with production of fababean at acid prone area is not well solved rather than lime amelioration. Hence, these researches were done to solve this existing problem. Accordingly, the highest adjusted grain yield was obtained from isolate FB-NS-03 as compared to inorganic fertilizer and other isolates. This isolate is better for future usage as overall. As additional information, the introduction of new rhizobia isolate from other area hadn't brought significant effects on this acid prone area. Therefore, isolation and evaluation of native rhizo-bacteria in the study area will might be bring the expected output.

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Conflicts of Interest

The authors declare no conflict of interest.

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